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Environmental PAH Exposure and Male Idiopathic Infertility: A Review on Early Life Exposures and Adult Diagnosis

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Abstract

The male reproductive system is acutely and uniquely sensitive to a variety of toxicities, including those induced by environmental pollutants throughout the lifespan. Early life hormonal and morphological development results in several especially sensitive critical windows of toxicity risk associated with lifelong decreased reproductive health and fitness. Male factor infertility can account for over 40% of infertility in couples seeking treatment, and 44% of infertile men are diagnosed with idiopathic male infertility. Environmental exposures are poorly characterized and understood due to limited data human intake. The latency between maternal and *in utero* exposure and a diagnosis in adulthood complicates the correlation between environmental exposures and infertility. The results from this review include recommendations for more and region specific monitoring of polycyclic aromatic hydrocarbon (PAH) exposure from the diet and air and longitudinal, clinical cohort considerations of exposure normalization, gene-environment interactions, and fetal origins of adult onset diseases, and controlled mechanistic animal experiments. Additionally, it is recommended that detailed semen analysis and male fertility data be included as endpoints in environmental exposure cohort studies due to the sensitivity of the male reproductive system to environmental pollutants, including PAHs.

Keywords

Polycyclic Aromatic Hydrocarbons; Male Idiopathic Infertility; Fertility; Environmental Pollution; Reactive Oxygen Species

Introduction

The average age of first time fathers is 24.5 years old in the Unites States [1]. Prior to attempted conception, infertility is not readily diagnosed, complicating the identification of

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environmental causes by the elapse of time between exposure and diagnosis. In mammals, sexual dimorphism and morphologic development occurs primarily during gestation, a critically sensitive timeframe linked to the initiation of several diseases diagnosed in adulthood [2, 3]. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants with three potential routes of exposure (oral, dermal, inhalation), occurring in highest concentrations in industrially polluted environments [4]. PAHs cross the placenta and have been identified as endocrine disrupters, reproductive toxicants, neural toxicants and cancer agents [5–7]. Previously, maternal smoking during pregnancy has been identified as a risk factor in male infertility [8, 9]. Environmental PAH exposures can occur in differing routes, mixtures and concentrations from PAHs in cigarette smoke and are less well studied. The focus of this mini-review is on the current understanding of early life environmental PAH exposures and their effects on male idiopathic infertility, including key knowledge gaps. Knowledge gaps include; quantitative PAH exposure data, the effects and mechanism of prenatal PAH exposure toxicities in brain and reproductive tissues, and the lifelong reproductive effects following male *in utero* PAH exposures, as detected by sensitive and uniform subacute endpoints. Additionally, gene-environment interactions are necessary for susceptibility determination.

Male idiopathic infertility

Male idiopathic infertility is defined as the inability to conceive a child during two or more years of trying, often diagnosed in men based on low sperm count or poor sperm quality, with no identifiable morphological causes [10]. Of men seeking treatment for infertility, 44% of instances are diagnosed as idiopathic [11]. Idiopathic male infertility is caused by endocrine disruption as a result of exposure to environmental pollution, reactive oxygen species, or genetic and epigenetic abnormalities [10]. Mutations or polymorphisms in spermatogenesis candidate genes are associated with idiopathic forms of spermatogenic disturbances [12]. Other than Y-chromosome related gene mutations or polymorphisms, no clinically relevant genes have been implicated in male infertility [12–14]

Men who smoke, or are exposed to PAHs through occupation, have bulky PAH-DNA adducts in sperm, reduced sperm count and motility and alterations in morphology [15, 16]. Semen is used a biomarker of environmental pollutant exposure, including that of PAHs [9, 17, 18]. PAH metabolites and PAH-DNA adducts in semen can indicate a potential cause of male infertility/ reduced fertility, but depending upon the cell specific toxicities and timeframe of exposure, limiting further exposure to PAHs may not be sufficient to restore fertility.

Male *in utero* development

Reproductive system development in mammals relies upon carefully programmed hormone signaling *in utero* for sex determination and further masculinization or feminization of offspring, occurring in the genital tract and the brain. Androgen receptors (AR) and estrogen receptors (ER) are present early in fetal development, expressed in the mesenchyme of the early non-sexually dimorphic urogenital anlagen [19]. In male rat fetuses, the in vivo levels of testosterone, the most studied androgen, exceed that of females at 14 days post coitum

[20]. In mammalian males, androgen dependent mechanisms are responsible for the formation of the epididymis, ductus deferens, and seminal vesicle [21]. Paracrine interaction between mesenchyme (AR-positive) and epithelia (AR-negative) drives androgen dependent epithelial development in tissues such as the bladder, prostate, bulbourethral glands, urethra, and periurethral glands [22].

Gonadal steroids, such as estrogen, testosterone, and metabolites thereof, have a role in male and female dimorphic brain and neural development [23, 24]. Sex based brain dimorphism includes differences in brain region sizes, number of nerve cells, distribution of neurotransmitters, and development of behavior [23–26]. Testosterone and the testosterone metabolite estradiol, have been well documented to drive sexually dimorphic brain development and masculinization by organizing neural circuitry to promote behavior attributed to males [27–29].

Prior to sexually dimorphic gonad morphology or the detection of differential levels of fetal gonadal steroids (14 days post coitum), there is growing evidence that more genes are involved in sex-based fetal brain development [20]. In rat embryonic mesencephalic or diencephalic neurons harvested prior to 14 days post coitum and cultured *in vitro*, have sexually differential neural enzyme expression [20, 30, 31]. Microarray analysis of 10.5 day post-coital fetal brain tissue, identified several differentially expressed genes between the sexes [32]. The differential gene expression in brain prior to the release of gonadal steroids indicates that brain may play an early role in the further sexual dimorphism in morphology. This also indicates a potential for xenobiotic interruption in the process of brain sexual dimorphism, which could lead to downstream consequences in further reproductive morphological development of the fetus.

To the author's knowledge, studies have not demonstrated that PAHs are directly able to cross the fetal blood brain barrier (BBB) and there are few studies on PAH quantitation in brain tissue. However, the cellular junctions in the fetal BBB are known to be leaky toward lipophilic compounds, relative to those of mature adults, and PAHs have been found to cross the BBB in adults [7, 33, 34]. There is growing evidence that PAH exposure *in utero* and in early childhood results in a number of cognitive and intellectual disadvantages [7, 35–37]. Androgen and estrogen receptors are present in the brain and brain development is affected by levels of sex steroids present in the fetal environment [38]. PAHs can bind to androgen and estrogen receptors [39, 40]. Together, the published literature indicates that PAHs are capable of crossing the placenta, crossing the fetal BBB, generating brain/neural toxicities, and potentially affecting sexual development of the brain and the gonad.

In utero PAH exposure

PAHs are documented to cross the placenta and to have been detected in fetal tissues [41, 42]. Maternal PAH exposure effects the fetus and fetal environment in several ways, including increased DNA strand breaks [43], aneuploidy via blockage of meiosis [44], and increasing sister chromatid exchange with increasing dose [45]. *In utero* PAH effects on subsequent adult male fertility, assessed from fetal exposure to mother's smoking, include decreased testicle size [46], and decreased sperm count [46, 47]. Paternal smoking during

the conception time frame is found to reduce the number of male children born relative to those born to non-smoking fathers [48, 49]. Animal models of *in utero* PAH exposure have reduced fertility, sperm count, and testicle size, or increased pro-apoptic protein expression [50–53]. Detecting PAH and PAH adducts in cord blood of neonates relative to PAH concentration in the mother, and at times pared environmental monitor data for PAH exposures, has been a recent trend in *in utero* PAH exposure studies, but often the endpoints include asthma and cognitive abilities, as mentioned previously [54–56]. Longitudinal studies would be necessary to determine the effects of these early life exposures on subsequent adult male reproductive health.

PAH Exposures

Environmental exposure to PAHs are not easily identifiable or modifiable, and can occur in different mixtures and at lower concentrations than those of cigarette smoke. In non-smokers, the dietary route of high molecular weight PAH exposure is often the greatest source, with approximately 95% obtained from the diet from a variety of foods including breads and cereals, grains, vegetables and smoke-cured or barbequed meats [4, 57, 58]. It is estimated that in the United States, total PAH intake for a non-smoking male from 19–50 years old is 3120 ng/day, of which 96.2% is from the diet [5]. PAH exposure through the diet varies considerably by the regional point source of PAH contamination in food items; many countries and regions are lacking data to accurately report intake of PAHs from food items [59]. The global trade of potentially high PAH concentration food items, such as olive oil, further complicates PAH intake estimation [60]. To understand regional dietary exposure to PAHs and the potential health risks, more research is necessary in the detection of PAHs from human foodstuffs. Given the long latency period between potential *in utero* PAH exposure and a diagnosis of associated adult onset diseases, including male infertility, regular monitoring for the PAH concentrations of regionally consumed foods would improve the abilities of epidemiological studies for potential correlation.

Another route of exposure to PAHs is inhalation of atmospheric PAHs from air pollution, such as near industrial or high automotive traffic areas and housing with inadequate ventilation of PAHs generated in heating or cooking [61–63]. Cooking over a solid fuel burning cook stove is often studied in developing countries, in households where women are responsible for the majority of cooking [64]. Cooking duties continue through pregnancy and small children often assist, increasing potential *in utero* and early life exposure to PAHs. Male infertility data from developing countries are rare, making correlation to PAH exposure difficult, if not impossible. PAHs bound to particulate matter from diesel exhaust is another source of inhalation exposure [65]. Additionally, atmospheric PAHs have been shown to migrate great distances to dispose into environments far from point source contamination [66]. Research on the transport, fate and toxicities of atmospheric PAHs has recently focused on weathering of PAHs to nitro- or oxy- PAHs [67]. It should be noted that nitro- or oxy-functionalized PAHs may be direct carcinogens, not pro-carcinogens, which would require enzymatic activation, leading to an increase in mutagenicity and radical formation during redox cycling with functionalizion [67–69]. There is limited data available on the effects of nitro- or oxy- PAH exposure on mammalian reproductive toxicities or the effects of *in utero* exposures.

Much of our current understanding of industrial exposure to PAHs, and the resulting effects on male fertility was established by the Teplice Program in the Czech Republic during the 1990s [70, 71]. This program followed individuals exposed to PAHs through industrial coal burning emissions and the subsequent longitudinal health results of reducing pollutant emissions. This was one of the first, and still one of the few, studies including the metrics of male fertility from a population based environmental pollutant exposure cohort. Young men directly exposed to relatively low concentrations of coal emissions (40 μg PM_{10}/m^3) were found to have significant toxic effects in sperm morphology, head shape, motility and aneuploidy, relative to control populations [72]. Exposure during the first month of gestation was the most significant with regard to reduced birth weight and intrauterine growth retardation [72, 73]. Follow-up studies confirmed previous findings, but also found that there was no significant difference in overall sperm concentration between exposure groups [74]. This indicates the need for detailed analysis beyond simple sperm counts from sub-acute exposure samples. The Teplice Program identified reproductive toxicities in men directly exposed to air pollution and reduced birth weight in neonates exposed *in utero*, but it did not follow up with the low birth weight babies to determine if they experienced sperm abnormalities or reduced fertility as adult men, who would now be approximately 25 years old. Currently, Central and Eastern Europe still have one of the highest global infertility rates, with 20% of couples reporting infertility in Poland, of those 40–60% are attributable to the male factor alone [75–77]. Data from well characterized exposures, such as those from the Teplice Program, and the effects on adult male fertility from *in utero* exposures are necessary for risk assessment due to the unique sensitivity of the developing fetus.

Mechanism

In addition to the previously mentioned steroid receptor mediated PAH pathways, PAHs are extensively metabolized *in vivo*. Parent PAHs are pro-carcinogens, cytochrome P450 (CYP) enzymes 1A1 and 1B1 activate the parent PAH to reactive diol and epoxide species which then adduct to DNA with specification for GC nucleotides [78, 79]. CYP1A1, and polymorphism thereof, have been indicated in numerous diseases of environmental origin, such as cancer [80]. The suspected mechanism is the P450 activation of PAHs to reactive intermediates that are then capable of binding to DNA to induce mutagenesis and cancer [71, 79]. Studies on loss or reduction of functional CYP1A1, related to genetic polymorphisms, the primary PAH metabolic enzyme, has produced conflicting results. The allelic variant, CYP1A1*2A, was found to be both protective and not protective of male idiopathic infertility relative to the wild type CYP1A1 genotype in humans [81–83]. The CYP1A1*2C allelic variant significantly reduced the risk of infertility more consistently [82, 84].

The contradictory results in linking P450 polymorphic status to infertility is likely related to the different levels of PAH exposure between the study participants [85]. Matched cohorts of CYP1A1*2C polymorphic and CYP1A1 wildtype volunteers that excluded or did not stratify by smoking status resulted in a false negative correlation between CYP1A1 status and infertility risk. Risk became significant when smokers were included in both cohorts and accordingly statistically corrected [85]. This highlights the importance of considering gene-environment interactions in the design of PAH exposure cohort studies and the necessity of

including cohorts with similar exposure patterns. Possible tools to characterize exposures to normalize cohorts include the usage of questionnaires, location to point source of PAH emissions, or environmental monitoring data, such as that detected by a recently developed personal exposure monitor [86].

Reactive oxygen species (ROS) can result from PAH dihydrodiols entering a redox cycle via aldo-keto reductase [87], while conjugation and urine elimination removes reactive radical intermediates from the redox cycle [88]. ROS must be carefully regulated in sperm and seminiferous fluid as sperm create ROS to assist in oocyte penetration, but are subject to DNA damage and reduced fecundity from excess ROS [89]. ROS in sperm and seminal fluid is associated with male idiopathic infertility [89, 90]. However, it is difficult to establish direct cause and effect from clinical and epidemiological studies. The extent of ROS generated by PAH metabolism is dictated by the extent of activation by P450s, the reactive intermediate formation by aldo-keto reductase, the rate of functionalized PAH elimination by glutathione S-transferases and UDP-glucuronosyltransferase, and finally the enzymatic antioxidant activity of superoxide dismutase or glutathione peroxidase [91]. To complicate matters, all of the before mentioned enzyme systems have human allelic variants, and seminal fluid contains dietary sources of antioxidants (ascorbic acid, α-tocopherol, carotenoids, and flavonoids) [92, 93]. Understanding *in utero* risks of PAH exposures, and potential long-term effects, is challenging because the expression of PAH metabolizing enzymes varies by organ and stage of development [94].

PAHs from cigarette smoke induces regulatory changes in PAH metabolic enzyme expression and a decrease in antioxidant concentrations [80, 95, 96]. In smokers, there is a 107% increase in seminal ROS levels [97] and in controlled animal studies of *in utero* PAH exposure, PAH generated ROS [8]. PAH detoxification by glutathione conjugation was inhibited in a mouse model null for glutamate cysteine ligase (Gclm), the rate-limiting enzyme in glutathione synthesis. When exposed prenatally to the PAH benzo[a]pyrene (BaP), the 10 week old Gclm knock out mice had reductions in: testicular weight, testicular sperm head counts, epididymal sperm counts, and epididymal sperm motility, relative to wildtype littermates also exposed to BaP [8]. This provides evidence that PAH exposure can generate ROS in the testis *in utero*, with consequences lasting into sexual maturity. Further controlled animal model studies are necessary to determine the reproductive effects in adult males from timed, low dose, chronic, *in utero* exposures, preferably utilizing models of differing polymorphic statuses.

During semen analysis, the degree of oxidative damage can be qualitatively determined by morphological endpoints, such as, asthenozoospermia, hyperviscosisty, and the increased presence of seminal leukocytes [98–101]. These methods are relatively simple and inexpensive, providing supporting information to clinical studies in lieu of more advanced quantitative methods. A quantitative approach, however, would allow more precise correlation to PAH exposure data and antioxidant intake. Measurement of malondialdehyde (MDA), a marker of sperm membrane peroxidation, can be detected by HPLC with fluorescence detection in sperm or seminal plasma [102, 103]. Sperm DNA oxidative damage can be measured directly in sperm or in seminal plasma by the HPLC detection of 8-hydroxy-2′–deoxyguanosine (8-OHdG) [95]. Total antioxidant capacity (TAC) should be

detected by spectrophotometric assays in conjunction with ROS detection to generate an index for the ability to neutralize ROS being generated in the system and to identify individuals who may benefit from antioxidant supplementation [104]. Inclusion of semen analysis as an additional parameter in environmental exposure cohort studies would further the field of environmental pollutant driven male infertility and would provide a tissue that is very sensitive to sub-acute exposure.

Therapy

Antioxidants administered to men with idiopathic infertility, in combination with *in vitro* fertilization, increased birth success rates [103]. However, the lack of consistent and reproducible results, as well as the variety of potential therapies, prevents the reliance on antioxidants as a standalone therapy [10]. The inconsistent study results have been attributed, in multiple review papers, to poor study design, the variety of antioxidant therapies used, short duration, and the potential differing underlying mechanism of infertility [90, 101, 104, 105]. The degree of PAH exposure and timing of exposure can initiate toxicities that are not limited to the germ cell [53, 106]. Idiopathic infertility has been associated with germ cell damage with, or without, Sertoli cell damage and there are differences in the degree of ROS or TAC among study participants, highlighting the need for carefully controlled study cohorts in clinical trials and quantitative measurements [104, 106]. Animal models have previously been used for antioxidant amelioration studies of PAH induced infertility [107], which could have utility in *in utero* or co-exposure models for prevention of PAH induced testicular ROS, but not for therapeutics in men currently experiencing infertility.

Conclusion

In humans, the *in utero* effects of exposures to a characterized PAH mixture and the subsequent effects on adult male infertility are limited. Males are found to be solely responsible for 20–30% of infertility cases and contribute to 50% of cases overall [75], and data is mostly limited to Western countries due to lack of research in developing countries. The social stigma attributed to the condition and limited availability or expense associated with seeking medical treatment limit incidence reporting. Developmental and tissue specific expression and gene-environment interactions make *in utero* PAH exposure risks difficult to assess. The long latency period between *in utero* exposures and a diagnosis of male idiopathic infertility and the lack of regional exposure data over time makes correlation to environmental pollutant exposure especially challenging.

There is evidence that fetal exposure to PAHs result in neural and reproductive toxicities and PAHs are known to be fetal toxicicants [46, 50, 94], but more research is necessary on the adult male fertility effects and the mechanism of toxicity. Further animal model research would permit controlled studies on the variables of timed exposures, characterized PAH exposures including functionalized PAHs (nitro- and oxy-) or mixtures exposures, dose/response, and gene-environment interactions. Additionally, well designed, human relevant studies of antioxidant therapies in animal models would be greatly helpful to the assessment of testicular damage prevention from ROS in *in utero* via co-exposures of maternal

antioxidants and PAHs, including endpoints into male sexual maturity and reproductive success. The efficacy of antioxidant therapy for *in utero* exposure generated adult male idiopathic infertility and the reduction ROS would also be useful data from such studies in animal models.

Quantitative analyses are necessary for the characterization of PAH exposure by location and the effects in semen related to male infertility and subfertility. Accurate quantitative methods are needed for exposure assessment, ADME in the fetus, and in the resulting biological endpoints in fetal and adult male tissues, including semen, gonad and brain from research models and available human samples. Semen quantitative endpoints, such as ROS, TAC, and DNA damage, are necessary to determine the subtle effects of low environmental dose exposure and potential therapies. The analysis of PAH content and profiles in foodstuffs and local eating habits over time are necessary due to the long latency period between *in utero* and early life exposures and adult onset disease diagnosis. Additionally, monitoring of regional PAH concentration in foodstuffs will provide a more accurate basis for dietary recommendations. Exposures to atmospheric PAHs can be periodically taken by air monitors or personal exposure monitors, generating data that can be used to stratify environmental PAH exposure cohorts.

Risk assessment and emissions regulation are designed to be protective of the most vulnerable populations, which for PAHs that cross the placenta, are arguably neonates [36, 50]. However, without reliable data on sensitive non-mortal endpoints, such as fertility, it is difficult to validate the safety of exposure recommendations. Translation of data from animal studies relies on carefully conducted human cohort studies, such as those of the Teplice Program [70]. When possible, semen analysis and male fertility data should be included in population cohort studies from high environmental PAH exposure regions. Health disparities research demonstrates that individuals of low socio-economic status have a higher incidence of smoking, smoking while pregnant, and living in areas susceptible to PAH air pollution, such as near highways and in industrial areas [61, 108–110]. Often minority populations are acutely affected by low socio-economic status and health care disparities, relative to the general population [111], indicating that PAH exposure related effects are of concern in populations traditionally underrepresented in research studies. It is critical that polymorphisms and gene-environment interactions are considered in further studies of PAH exposures.

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